

## Protective Action of Plant Polyphenols on Radiation-Induced Chromatid Breaks in Cultured Human Cells

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**Abstract.** The present study was performed to determine whether plant polyphenols can protect human cells against radiation-induced DNA damage manifested as chromatid breaks. Since each chromatid contains a single continuous molecule of double-stranded DNA, chromatid breaks represent unrepaired DNA strand breaks. The addition of green or black tea extracts, their polyphenols or curcumin to cultures of human skin fibroblasts or PHA-stimulated blood lymphocytes significantly reduced the frequencies of radiation-induced chromatid breaks. An exception to this general finding was that the green tea polyphenol, (-)epigallocatechin gallate, had no effect. The protective action of these plant polyphenols seems to result from their known antioxidant properties, particularly the scavenging of hydroxyl free radicals. Frequencies of chromatid breaks in cells arrested immediately after irradiation or 0.5 to 1.5 hours post-irradiation in the presence or absence of a DNA repair inhibitor, provide a measure of DNA damage. The results of the present study show that tea and other plant polyphenols can protect human cells against radiation-induced DNA damage.

Leaves of the tea plant *Camellia sinensis* contain specific polyphenols and an enzyme polyphenol oxidase. The primary polyphenols in black and green tea include theaflavin gallates

and (-)epigallocatechin gallate (EGCG) respectively, depending on the activation of polyphenol oxidase during processing (1). Additionally the rhizome of the plant *Curcuma longa* contains another polyphenol, curcumin (2). These polyphenols provide antioxidant potentials which inhibit carcinogenesis in animal models (3-5), decrease genotoxicity in the *Salmonella typhimurium* test of Ames and inhibit mutagenicity in Ames and Williams tests (6). In animals, and to some extent humans the inhibition of carcinogenesis with tea polyphenols correlates with a lower level of 8-OH-dG in DNA. This observation suggests that the inhibitory effect of tea compounds on carcinogenesis results from reducing the interaction of reactive oxygen compounds with DNA (6). Chromosomal aberrations have been used as sensitive indicators of DNA damage and repair for the past 20 years (7, 8), and chromatid breaks reflect genotoxic effects in clastogenic assays (9). Since each chromatid of the metaphase chromosome contains a single continuous DNA molecule, chromatid breaks represent unrepaired DNA strand breaks. These can arise directly from a damaging agent or from enzymatic incision during the repair process. In the present study we intended to examine the protective action of plant polyphenols on radiation (x-ray and fluorescent light)-induced DNA damage manifested as chromatid breaks in normal human cells in culture. Since DNA lesions leading to chromatid breaks are rapidly repaired in such cells, a repair inhibitor, 1- $\beta$ -D-arabino-furanosylcytosine (ara-C) was added to certain cultures to block repair (10) in order to assess the DNA damage and the protective action of the plant products.

**Abbreviations used:** ara-C, 1- $\beta$ -D arabinofuranosylcytosine; BTE, black tea extract, BTP black tea polyphenols; CBF, chromatid break frequency; EGCG, (-)epigallocatechin gallate, GTE, green tea extract; GTP, green tea polyphenols; TFM, theaflavin mixture.

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### Materials and Methods

**Cell source and culture procedure.** Lines of skin fibroblasts, GM 5565 and GM 5757 were obtained from the Coriell Institute of Medical Research, Camden, NJ. Cells were grown in Dulbecco's modification of Eagle's MEM supplemented with 10% fetal bovine serum (Flow Laboratories, McLean, VA) by methods described previously (11). Samples of blood from healthy laboratory workers were obtained and PHA-stimulated cultures of whole blood were established as described (12).

Table I. Effect of green tea polyphenols (GTP), black tea polyphenols (BTP), and (-) epigallocatechingallate (EGCG) on X-ray (53 rads)-induced chromatid breaks in human fibroblast cell lines.

Treatment	Chromatid breaks/ 100 Metaphase cells ( $\pm$ S.E.)	
	Line GM 5565	Line GM 5757
None	1.5 ( $\pm$ 1.5)	2.0 ( $\pm$ 1.4)
X-ray alone	80.0 ( $\pm$ 11.7)	56.0 ( $\pm$ 7.7)
X-ray + GTP	12.9 ( $\pm$ 3.7) $p < 10^{-5}$ *	9.8 ( $\pm$ 3.1) $p < 10^{-5}$ *
X-ray + BTP	16.7 ( $\pm$ 4.9) $p < 10^{-4}$ *	10.4 ( $\pm$ 4.0) $p < 10^{-3}$ *
X-ray + EGCG	93.6 ( $\pm$ 14.6)	56.9 ( $\pm$ 8.5)

\*Compared to X-ray alone

**Plant extract.** Extracts of black and green tea and their respective polyphenols including theaflavin mixture, and (-) epigallocatechin gallate as well as curcumin from turmeric were generously supplied by Dr. D.A. Balentine of the Thomas J. Lipton Company, Englewood Cliffs, NJ. The compositions and chemical structures of these compounds have been presented elsewhere (2, 6). The tea compounds were dissolved in 4X glass-distilled water and filter-sterilized. Curcumin was supplied as a 1% solution in a 50:50 mixture of polyethyleneglycol and glycerine.

**Experimental procedure.** Forty-eight hour cultures of skin fibroblasts grown on 9x50mm coverslips in Leighton tubes were x-irradiated (53 rads) as described (11). The culture medium was renewed immediately after x-irradiation and cells arrested in metaphase with colcemid (0.1  $\mu$ g/ml) from 0 to 0.5 hours post-irradiation. In certain cultures the tea components were added to the culture medium 1 hour before x-irradiation and again added to the medium at its renewal after irradiation.

To examine the effect of fluorescent light on PHA-stimulated peripheral blood lymphocytes, a 5 mL cell suspension equivalent to a hematocrit of 0.5 (12) was inoculated into each T-25 flask. These flasks of lymphocytes or 48 hour Leighton tube cultures of fibroblasts were exposed for 3 hours to cool-white fluorescent light (8 W/m<sup>2</sup>) in a horizontal position. Certain cultures as indicated received tea components or curcumin 1 hour before light exposure and/or 60  $\mu$ L of ara-C (1 mg/mL) 0.5 hour after exposure. Cells were arrested in metaphase by colcemid added 0.5 to 1.5 hours after light exposure. The fibroblasts grown on coverslips or blood cells in suspension were processed for chromosome analysis as described (11, 12).

All experiments were carried out in the "In Vitro Carcinogenesis Section" of the National Cancer Institute (NCI). The coded preparations were analyzed for DNA damage at Howard University and decoded at NCI after the data had been tabulated. Fifty to 100 metaphase cells were examined per variable. Aberrations scored as chromatid breaks showed either non-alignment and displacement of the broken segment (displaced breaks), or if non-displaced, a discontinuity longer than the chromatid width. Each set of data on skin fibroblasts and blood lymphocytes is based on quadruplicate and duplicate sets of cultures respectively. Chromatid break frequencies were compared using the chi-squared test for comparing multinomial distributions or Fisher's exact test (13).

## Results

### Effects of tea polyphenols on X-ray-induced chromatid breaks in

Table II. Effect of black tea extract (BTE), black tea polyphenols (BTP), green tea extract (GTE), green tea polyphenols (GTP), theaflavin mixture (TFM) and (-) epigallocatechin gallate (EGCG) on fluorescent light (FL)-induced chromatid breaks in human cells in the presence or absence of ara-C in PHA-stimulated blood lymphocytes from normal donors.

Treatment	Chromatid breaks / 100 Metaphase cells ( $\pm$ S.E.)			
	Skin Fibroblasts GM 5757	Blood lymphocytes		
		Donor #1	Donor #2	Donor #3
FL alone	3.0( $\pm$ 17)	2.0( $\pm$ 2.0)	2.0( $\pm$ 2.0)	0
FL+BTE	-	2.0( $\pm$ 2.0)	-	-
FL+GTE	-	0	-	-
FL+TFM	-	-	2.0( $\pm$ 2.0)	2.0( $\pm$ 2.0)
FL+EGCG	-	-	2.0( $\pm$ 2.0)	2.0( $\pm$ 2.0)
FL+araC	34.0( $\pm$ 4.8)	32.0( $\pm$ 8.3)	28.0( $\pm$ 6.4)	32.0( $\pm$ 7.8)
FL+araC+BTP	7.0( $\pm$ 2.0)****	-	-	-
FL+araC+BTE	-	2.0( $\pm$ 2.0)**	-	-
FL+araC+GTP	19.3( $\pm$ 3.5) *	-	-	-
FL+araC+GTE	-	0***	-	-
FL+araC+TFM	-	-	2.0( $\pm$ 2.0)**	2.0( $\pm$ 2.0)**
FL+araC+EGCG	45.1( $\pm$ 4.6)	-	30.0( $\pm$ 8.2)	34.0( $\pm$ 6.8)

When compared to FL+araC: \* $p < 10^{-2}$ , \*\* $p < 10^{-3}$ , \*\*\* $p < 10^{-4}$ , \*\*\*\* $p < 10^{-5}$

**fibroblasts.** Table I shows the effects of GTP, BTP, and EGCG on x-ray-induced chromatid breaks in two lines of human skin fibroblasts. X-irradiation (53 rads) produced chromatid break frequencies (CBFs) of 80 and 56 in cells of lines GM 5565 and 5757 respectively. The addition of GTP or BTP to the culture medium 1 hour before x-irradiation significantly reduced these CBFs. EGCG, on the other hand, did not affect the x-ray-induced CBFs in either cell line. GTP or BTP might produce a mitotic block resulting in an accumulation of metaphase cells during the hour prior to x-irradiation. Cells in metaphase at the time of irradiation do not show radiation-induced chromatid breaks (Unpublished observations), and thus could give the false impression of having been protected by GTP or BTP. We counted the number of cells in metaphase in cultures irradiated or not irradiated in the presence of GTP or EGCG. The ratios of metaphase cell numbers in irradiated compared to nonirradiated cultures were equivalent after treatment with GTP or EGCG, i.e. 0.965 and 0.933 respectively. These data show that reduction of CBF in the presence of the tea polyphenols, GTP does not result from a mitotic block. The

reduction thus appears to result from their radioprotective actions.

**Effects of tea extracts and polyphenols on fluorescent light-induced chromatid breaks.** Normal human cells proficiently repair the light-induced DNA damage that leads to chromatid breaks. Therefore, to determine the extent of light-induced damage, repair was inhibited in all subsequent experiments by adding ara-C to the culture medium 0.5 to 1.5 hours after light exposure. Under these conditions fluorescent light produced a CBF of 34 in skin fibroblasts of the cell line, GM5757 (Table II). Addition of GTP or BTP to the culture medium 1 hour before light exposure reduced the CBF to 19 and 9 respectively. This reduction was statistically significant ( $p < 0.008$  and  $p < 10^{-5}$  for GTP and BTP respectively). EGCG, on the other hand, had no effect on CBF.

Table II further shows the effects of BTE, GTE, and their primary polyphenols, TFM and EGCG respectively, on PHA-stimulated peripheral blood lymphocytes from three different donors. Light exposure with or without added tea extracts or their polyphenols yielded a low CBF of  $\leq 2$ . This CBF was increased to 28 or 32 when ara-C was added after light exposure. Addition of BTE, GTE, or TFM reduced this CBF to 2, 0, and 2, respectively. EGCG on the other hand had no effect.

Table III shows the effect of curcumin on fluorescent light-induced chromatid breaks in PHA-stimulated peripheral blood lymphocytes from two donors. Light exposure produced no chromatid breaks in cells of donor No. 1. Addition of ara-C increased the CBF to 84 and 60 in cells of donors Nos. 1 and 2, respectively. Curcumin at a concentration of 100  $\mu\text{g/ml}$  inhibited mitosis in the presence or absence of light exposure. Increasing the concentration of curcumin from 10 to 80  $\mu\text{g/ml}$  lowered the CBF in a dose-related manner.

## Discussion

The present study shows that the addition of green or black tea extracts, their respective polyphenols, or theaflavin mixture significantly reduced the frequencies of x-ray-induced chromatid breaks in human skin fibroblasts. The effects of these compounds were seen in cells entering metaphase 0-0.5 hours post irradiation so that the cellular DNA was damaged in late prophase or  $G_2$  phase of the cell cycle. The reduction of chromatid break frequency could result from radiation-induced cell death or cell cycle disturbance. However, we have previously shown that x-irradiation or fluorescent light exposure at the doses used do not produce cell death or cell cycle delay (14 - 16). We reported previously (17) that mannitol, a scavenger of the reactive free hydroxyl radical ( $\bullet\text{OH}$ ) or catalase, which decomposes hydrogen peroxide, when added to the culture medium during or after x-irradiation decreased CBF in a dose-related manner. X-rays are known to generate  $\bullet\text{OH}$ , through radiolysis of water (18). These observations suggest

Table III. Effect of curcumin (Cu) on fluorescent light (FL)-induced chromatid breaks in the presence of ara-C in PHA-stimulated blood lymphocytes from two normal donors.

Treatment	Chromatid breaks / 100 Metaphase cells ( $\pm$ S.E.)	
	Donor #1	Donor #2
FL+araC	84.0( $\pm$ 19.2)	60.0( $\pm$ 14.6)
FL+araC+Cu(10 $\mu\text{g/ml}$ )	88.0( $\pm$ 18.7)	-
FL+araC+Cu(20 $\mu\text{g/ml}$ )	68.0( $\pm$ 13.8) $p < 0.46^*$	-
FL+araC+Cu(40 $\mu\text{g/ml}$ )	34.0( $\pm$ 7.9) $p < 0.048^*$	32.0( $\pm$ 8.3) $p < 0.38^*$
FL+araC+Cu(60 $\mu\text{g/ml}$ )	-	18.0( $\pm$ 5.5) $p < 0.017^*$
FL+araC+Cu(80 $\mu\text{g/ml}$ )	-	8.0( $\pm$ 3.9) $p < 0.001^*$

\*Compared to FL+araC

that the protection afforded by the tea components results from their action as free radical scavengers. Steele *et al* (19) reported that most of the tea fractions used here inhibited TPA-induced free radicals in human HL60 cells. However, EGCG, as observed in the present study, was ineffective (see footnotes), possibly because of inadequate uptake and/or retention in the cells. The chromatid break assay can thus be used to measure the protective action of chemopreventive agents against DNA damage.

The sensitivity of this chromatid break assay for DNA damage during  $G_2$  can be greatly increased by DNA repair inhibitors such as caffeine or ara-C, both of which enhance the effects of mutagens (10, 20). Although the effect of caffeine on DNA repair during  $G_2$  is not well defined, ara-C blocks the polymerase step in excision repair and results in the accumulation of unligated DNA strand breaks which are processed into chromatid breaks seen at the subsequent metaphase (10, 21). The ara-C effect on the frequency of chromatid breaks thus reveals the enzymatic incision activity at damaged sites in DNA. The absence of an ara-C effect on CBF in cells exposed to fluorescent light in the presence of plant polyphenols (except EGCG) reflects the lack of damaged sites due to their DNA-protecting action.

The prevention of DNA damage may well be of prime importance in the prevention or blocking of the carcinogenic process in humans. Such activities at the molecular and cellular level could importantly prevent or delay carcinoma onset.

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